

News

Table 1. Breakdown of expressed sequence tags generated to date for *Schistosoma mansoni**

EST category	Number	Range across libraries (%)
Match with existing <i>Schistosoma</i> genes	623	6-32
Match with existing sequence from another species	551	5-43
No significant database match	1517	32-71
No breakdown available	188	
Total	2879	

*The overall efficiency of EST generation has been 83%, with a gene discovery rate of 60%. In addition, new isotypes of known *Schistosoma* genes have been detected.

cosmid and YAC probes, has been optimized⁵. However, these techniques are time-consuming, and small fragments (eg. cDNA clones) cannot be used as probes. The primed *in situ* technique (PRINS), which is simple, rapid and able to localize small fragments, has therefore been adapted for use with schistosome chromosomes (Hiro Hirai, Kyoto University, Japan). Individual chromosomes have been isolated from chromosome spreads by microdissection and the recovered DNA shown to be suitable for molecular manipulation (H. Hirai).

Genome Informatics

In common with the other WHO Parasite Genome Initiatives, an ACEDB-

based genome database will be produced for *Schistosoma*. An initial release, for local installation, is planned for February 1997 and will be available by FTP from the European Bioinformatics Institute (EBI) (<ftp://ebi.ac.uk/pub/databases/parasites/Schisto>). A WWW interface to allow querying of the master database is scheduled for release in Spring 1997. Database distribution will also be by CD-ROM. A WWW and gopher server, holding schistosome genome-related material, is under development. Announcements detailing the release of these informatics resources will be made on the Bionet schisto, parasitology and parasite-genome Newsgroups. Co-ordination and development of genome informatics is

the responsibility of the Network Secretary (D.A. Johnston, The Natural History Museum, London, UK) to whom inquiries should be addressed.

Acknowledgements

Juçara Carvalho-Parrá (Centro de Pesquisas René Rachou, Brazil) assembled the SGN in Ouro Preto, Brazil 25-27 June 1996, with joint WHO/FIOCRUZ (Fundação Oswaldo Cruz, Ministry of Health, Brazil) sponsorship, to evaluate the current status of the WHO/UNDP/World Bank *Schistosoma* Genome Initiative. A full report from this Meeting, including contact addresses for all groups, is available at: <http://www.nhm.ac.uk/schisto/who/meetings/96meet/96meet.html>.

References

- 1 Franco, G.R. et al. (1995) *Gene* 152, 141-147
- 2 Tanaka, M. et al. (1995) *Mol. Biochem. Parasitol.* 69, 41-51
- 3 Robinson, D. et al. *Int. J. Parasitol.* (in press)
- 4 Adams, M.D. et al. (1991) *Science* 252, 1651-1656
- 5 Tanaka, M. et al. in *Genes and Antigens of Parasites: A Laboratory Manual* Vol. 2, FIOCRUZ (in press)
- 6 Tanaka, M. et al. *Mem. Inst. Oswaldo Cruz* (in press)
- 7 Hirai, H. and LoVerde, P.T. (1995) *Parasitol. Today* 11, 310-314

David Johnston is at the Biomedical Parasitology Division, The Natural History Museum, London, UK SW7 5BD. Tel: +44 171 938 9297, Fax: +44 171 938 8754, e-mail: daj@nhm.ac.uk

Comment

The Need for Assays Predictive of Protection in Development of Malaria Bloodstage Vaccines

L.H. Miller, M.F. Good and D.C. Kaslow

Asexual erythrocytic (bloodstage) parasites cause malaria, the disease associated with plasmodial infection. The goal of vaccines against bloodstage parasites is the prevention or reduction of disease and death in developing and newly industrialized tropical countries, especially in Africa. Because of the terrible toll of sickness and death, and because of the escalating resistance of malaria parasites to chloroquine and other drugs, there is a great sense of urgency to develop vaccines that can be tested in humans.

The candidate bloodstage vaccine for which we currently have the most

information is the polymerized synthetic peptide vaccine, referred to as SPf66. The vaccine has been given to thousands of volunteers in South America, Africa and Southeast Asia¹⁻³. Because of the use of surrogate end points such as fever or a particular parasitemia and because of large confidence intervals² or lack of efficacy³, there is still uncertainty as to whether SPf66 is efficacious in reducing severe disease and death in Africa. The authors of a trial of SPf66 in Tanzania² argue for the need for investigations to understand better the mechanisms involved with the aim of improving

efficacy¹. We agree that understanding immune mechanisms and assays would be essential to accelerate development of all bloodstage vaccines.

Two approaches to develop vaccines against the bloodstages of malaria parasites are (1) the reduction or elimination of erythrocytic parasites and (2) the understanding of disease pathogenesis⁴ and development of methods specifically to block these complications. The discussion that follows focuses on the former approach.

Essential criteria for a successful vaccine are: (1) target epitopes that have limited or no polymorphism; (2) a

Comment

human-compatible adjuvant; and (3) a strategy to successfully identify such an antigen/adjuvant formulation for manufacture and further development. Although none of the current candidate immunogens fulfills all these criteria, a few recombinant *P. falciparum* immunogens from regions of RESA (ring-stage erythrocyte surface antigen)⁵, SERA (serum-rich antigen)⁶ and merozoite surface protein 1 (MSP1)^{7,8} have shown promise in vaccine trials in New World monkeys (*Aotus* and *Saimiri*).

The unlimited supply of mice, and the availability of four species of rodent malaria parasites, have proven invaluable in the development of human malaria vaccines. For example, mice vaccinated with MSP1 of *P. yoelii* were protected against a lethal *P. yoelii* infection⁹. Vaccination with the C-terminus of *P. yoelii* MSP1 induced protective immunity^{10,11}. As a result of the success in rodents, the highly conserved C-terminus of MSP1 became the focus for development of recombinant *P. falciparum* antigens for testing in New World monkeys.

However, to date, protective immunogens appear to require complete Freund's adjuvant (CFA); other adjuvants suitable for human use such as alum have not been effective. Mice have been protected following vaccination with MSP1 constructs with different adjuvants¹², but it is unknown whether *P. falciparum* MSP1 in combination with these adjuvants will lead to protection of humans against *P. falciparum*. Because CFA is unacceptable for use in humans, it may be necessary to define the role of CFA and attempt to mimic its precise activities with other adjuvants or cytokines or use BCG, which is closely related to the active moiety in CFA and which can be transformed with foreign genes.

The vaccine carrier used may influence the efficacy. Multiple recombinant proteins have been synthesized that include the C-terminus of MSP1. One recombinant fusion protein included foreign T-cell helper sequences from tetanus toxin⁷; another (the 42 kDa C-terminus) included sequence upstream of the 19 kDa C-terminus to provide T-cell help for antibody production and possibly for cellular immunity⁸. These recombinant proteins need to be compared in the same trial.

When are the data adequate to proceed to human trials with a particular construct? It may not be possible to carry out challenge infections of normal volunteers after vaccination against bloodstages because of the potential

risk to the volunteers. Furthermore, candidate bloodstage vaccines can only be tested for their effects on disease and death in populations in endemic areas. The large expense of such vaccine trials demands that we attempt to predict which formulations of immunogen and adjuvant have the best chance of success.

Given the large number of variables, it is crucial to define immune effector mechanisms, and *in vitro* assays that correlate with protective immunity. The mechanisms of natural immunity to malaria models or the mechanisms by which recombinant antigens induce immunity are still unknown; these data would accelerate progress towards a vaccine and developing relevant *in vitro* assays. Although monoclonal antibodies (mAbs) have been important in defining potential vaccine targets, the mechanism of immunity induced by a recombinant antigen may differ from the action of the mAb. Effector mechanisms can most easily be studied in rodent malaria, where the tools (eg, knockout mice that lack B cells or Fc receptors) exist to dissect the relative roles of antibody [either neutralizing or antibody-dependent cellular immunity (ADCI)] and cell-mediated immunity (CMI).

The correlation of *in vitro* assays with protection can be determined using sera and peripheral blood cells from vaccinated mice and monkeys. Validation of such assays would require similar studies in human clinical trials. Possible serologic assays include blocking erythrocyte invasion and ADCI¹³ determined on *in vitro* cultured parasites, and fine specificity mapping and antibody isotype determined against peptides and recombinant antigens. That parasite-specific T-cell clones can adoptively transfer protection¹⁴ suggests that some parasite-specific antigens or epitopes can be targets of protective effector T cells. Human malaria-specific T cells can also inhibit growth of *P. falciparum* *in vitro*¹⁵, and malaria-specific T cells induced by vaccination may limit parasite growth *in vivo*.

At some point with any particular candidate, expensive field trials may have to proceed in the absence of assays predictive of protection. It would be tragic if a vaccine were delayed that might save lives. It would be equally tragic, however, with the limited resources available to malaria research, to proceed without putting some effort into understanding the mechanisms of protection and developing an assay to measure that effect. If we agree that

such assays would greatly assist in vaccine development and testing in the field and that the development of the assays is far less expensive than the field tests themselves, why are we willing to proceed without an attempt to obtain this information? Repeated failures in expensive field trials because of an uninformed choice of vaccines may discourage public funders and private industry from supporting future efforts to develop effective malaria vaccines. Despite these problems, the challenge is to bring to bear the power of modern biology to produce a safe and effective malaria vaccine that can be included in childhood vaccine in Africa and other affected countries.

References

- 1 Valero, M.V. et al. (1993) *Lancet* 341, 705-710.
- 2 Alonso, P.L. et al. (1994) *Lancet* 343, 1175-1181.
- 3 D'Alessandro, U. et al. (1995) *Lancet* 346, 462-467.
- 4 Miller, L.H., Good, M.F. and Mcken, G. (1994) *Science* 264, 1878-1883.
- 5 Collins, W.E. et al. (1986) *Nature* 322, 259-262.
- 6 Insunsa, J. et al. (1991) *Infect. Immun.* 59, 1247-1250.
- 7 Kumar, S. et al. (1995) *Mol. Med.* 1, 325-332.
- 8 Cheng, S.P. et al. (1996) *Infect. Immun.* 64, 253-261.
- 9 Holder, A.A. and Freeman, R.R. (1981) *Nature* 294, 361-366.
- 10 Daly, T.M. and Long, C.A. (1993) *Infect. Immun.* 61, 2462-2467.
- 11 Ling, I.T., Ogun, S.A. and Holder, A.A. (1994) *Parasite Immunol.* 16, 63.
- 12 Daly, T.M. and Long, C.A. (1996) *Infect. Immun.* 64, 2602-2608.
- 13 Bouharoun-Tayoun, H. et al. (1995) *J. E.p. Med.* 182, 407-417.
- 14 Burns, J.M. Jr et al. (1988) *Proc. Natl. Acad. Sci. U. S. A.* 85, 602-606.
- 15 Fell, A.H., Currier, J. and Good, M.F. (1994) *Parasite Immunol.* 16, 579-586.

Louis Miller and David Kaslow are at the Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA. Michael Good is at the Cooperative Research Centre for Vaccine Technology, Queensland Institute of Medical Research, 300 Herston Road, Brisbane, Australia 4029. Tel: +1 301 496 2183, Fax: +1 301 402 0079, e-mail: Louis.Miller@nih.gov

Indexes

A Subject Index and an Author Index for *Parasitology Today* 1996 is included in this (Feb '97) issue of *Parasitology Today*.